

Detection of BRDU in Formalin-Fixed, Paraffin Embedded Mouse Tissue

Reagent and Antibody Information

[1X Wash Buffer](#)

[3% Hydrogen Peroxide](#)

[1% BSA Diluent](#)

[2N Hydrochloric Acid](#)

[Boric Acid-Borate Buffer](#)

[Trypsin](#)

[DAB Chromagen](#)

[Hematoxylin](#)

Blocking Serum: Normal Rabbit Serum

Jackson ImmunoResearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Catalog # 011-000-001

Avidin / Biotin Blocking Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog # SP-2001

Primary Antibody: Rat Anti-BRDU

Accurate Chemical and Scientific Corp.

Westbury, NY 11590

www.accuratechemical.com

1-800-645-6264

Catalog # OBT0030

Secondary Antibody: Biotinylated Rabbit Anti-Rat IgG (H+L)

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog # BA-4001

Label Complex: Vectastain Elite ABC Kit (Standard)

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog # PK-6100

Staining Procedure

Positive Control Tissue: Tissue that has BRDU-labeled cells via BRDU incorporation into the animal
Stain localization: Nuclear

1. Deparaffinize and hydrate slides through the following solutions:

Solution	Repetitions	Time
Xylene	2 times	5 minutes
100% Ethanol	2 times	3 minutes
95% Ethanol	2 times	3 minutes
1X Wash Buffer	2 times	5 minutes

2. Place the slides in 2N hydrochloric acid for 20 minutes in a water bath at 37°C.
3. Place the slides in a boric acid-borate buffer solution for 1 minute at room temperature.
(Made by mixing 85ml of boric acid with 15ml of sodium biborate. Adjust the volume proportionately, if necessary.)
4. Proteolytic-Induced Epitope Retrieval Using Trypsin
Incubate the slides in a 0.01% trypsin solution in a water bath at 37°C for 3 minutes.
(DO NOT add the trypsin to the 0.05M Tris-HCl • CaCl₂ solution until 5 minutes prior to incubation.
Trypsin loses 75% of its reactivity within 30 minutes at 37°C.)
Rinse the slides in distilled water for 1 minute to stop the enzymatic digestion.
5. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.
6. Quench endogenous peroxidase by placing the slides in 3% hydrogen peroxide for 10 minutes.
7. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.
8. Block with 10% Normal Rabbit Serum for 20 minutes at room temperature.
Lot # _____ Date Reconstituted _____

DO NOT RINSE THE SLIDES. CONTINUE TO AVIDIN-BIOTIN BLOCK.

9. Avidin / Biotin Blocking Kit
Lot # _____ Exp Date _____ New Kit: yes / no
Apply avidin block for 15 minutes at room temperature.
Quick rinse in 1X Wash Buffer.
Apply biotin block for 15 minutes at room temperature.

DO NOT RINSE SECTIONS WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY. ONLY WIPE EXCESS BLOCK.

10. Apply the primary antibody at a 1:2000 dilution and incubate for 30 minutes at room temperature.
Lot # _____ Date Aliquoted _____

11. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.
12. Apply the rabbit anti-rat secondary antibody at a 1:500 dilution and incubate for 30 minutes at room temperature.
Lot #_____ Date Reconstituted_____
13. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.
14. Apply the label complex from the Standard Elite Kit and incubate for 30 minutes at room temperature.
15. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.
16. Apply the DAB chromagen and incubate in the dark for 6 minutes at room temperature.
(Add 1 drop of DAB per ml of substrate)
Lot #_____ Exp Date_____ New Kit: yes / no
17. Rinse the slides in tap water 3 minutes.
18. Counterstain with Harris Hematoxylin for **2 minutes**.
19. Rinse the slides in tap water until water is clear.
20. Gently agitate slides in 1X Wash Buffer until they turn blue.
21. Dehydrate through the following solutions:

95% Ethanol	1 time	3 minutes
100% Ethanol	3 times	3 minutes
Xylene	2 times	5 minutes
22. Coverslip

Updated 03/16/09